6. ANALYSIS OF THE HI-6, HS-3 and HS-6, INFLUENCE ON THE LIVER METHABOLIZING ENZIME SYSTEMS

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INTRODUCTION

At the present stage of the therapy and prophylactics of the poisonings with organophosphorous compounds (OPC), the reactivators of the cholinesterase take an important place. However, the application of these compounds is always combined with some other drugs. Therefore, it is necessary to know the possibilities for influence of the drug metabolism by the reactivators, as well their own metabolism and pharmacokinetics. This type of research is very necessary, when the newly synthesized compounds are concerned. Our research has shown that the classical reactivator of the cholinesterase Toxogonin, causes decrease of microsomal ethylmorphin N-demethylation activity, which probably was connected to the coincident loss of cytochrome P450 (Dishovsky, et al, 1989). The reactivators TMB-4 (Trimedoxime) and Diethyxime, have a similar effect (Dishovsky, et al, 1990). In this relation, the aim of the present work was to follow changes in liver drug metabolism after in vivo treatment with some H-Oximes – HS-3, HS-6 and HI-6, which are reactivators of the cholinesterase. This is necessary, in order to optimize the conditions for the application of these antidote compounds during the therapy and prophylactics of the OPC intoxications.

MATERIALS AND METODS

The experiments were performed on male albino Wister rats weighting 160-180 g. The animals were divided in groups of five. They were treated with the following cholinesterase reactivators: HS-6, HS-3 and HI-6. Each reactivator was injected i.m. in two different doses – 20 mg./kg. b. weight and 0,25mmol/kg b. weight for three different time intervals – 2nd, 24th hour after the injection and with the same dosages, injected daily, after three days. For hexobarbital sleeping time studies, animals were injected i.p. With 100 mg. b. weight hexobarbital, 30 min, after the injection of the reactivators.

For the biochemical studies rats were sacrificed and the livers were perfused with iced physiological solution, homogenized in isotonic solution of KCl (0,05M Tris-HCl, pH=7,4) and microsomic fraction was separated. The following parameters were measured:

- ethylmorphin N-demethylation activity (EMD) by the method of Nash (1953);
- aniline hydroxylation activity (AH) – by the method, modified by Mazel (1971);
- amaunts of cytochrome b5 and P-450 (Omura and Sato, 1964);

The protein content in microsomes was determined after Lowry et al. (1951) procedure. Results were statistically processed by Student's T-test.

RESULTS AND DISCUSSIONS

On Table 1 are presented the results from the effect of the researched cholinesterase reactivators on the length of the hexobarbital sleep of rats treated with 2 doses of the compounds and during various time intervals.

It was found that HS-3 shows tendency toward shortening of the sleeping time of the rats, when the observed interval was 2 hours. HI-6 - 0.25 mmol/kg dose also cause shortening of the hexobarbital sleep, while on the 72nd hour the two doses had a tendency toward a sleep time, longer than the control group. It was also found that the two applied HS-6 doses on the

2nd and 24th hour after the i.m. application did not show any statistically reliable influence on the length of the hexobarbital sleep in comparison with the respective control group.

The results of the research of the in-vivo injected cholinesterase reactivators effect on some parameters of the liver is shown on the following tables. On table 2 is shown the effect of the reactivators of the cholinesterase on the activity of the microsomal mixed function monooxygenases. The observation times were 2 hours after the application of a dose of 0.25 mmol/kg, b. weight. The experiment showed that neither of the researched reactivators of the cholinesterase had an effect on the liver drug metabolism on the 2nd hour after the application of the reactivator.

On the Table 3 is shown the effect of HS-6 and HS-3 on the monooxigenase activities enzyme on the 24th hour after the application of the compounds in a does of 0.25 mmol/kg b. weight. The experiment showed that 24 hours after its application HS-3 caused decrease of the activity of AH compared to the control group. Neither of the two observed cholinesterase reactivators had a major influence on the rest of the researched parameters.

On Table 4 is shown the effect of the researched compounds on the liver drug metabolism after a daily application of the reactivators for a three-day period in a dose of 0,25 mmol/kg. b. weight. The conclusion was that the HI-6 decreases to a large extent the activity of the AH.

In an enzyme-dependent metabolic transformation of the oximes (primarily in the liver) took part various enzyme systems. Our experiment data showed that even indirectly, the cytochrome P-450 system in the liver (of the experimental animals) was taking part in the metabolism of the oximes, or at least it was changing under the influence of the oximes and their metabolites.

Our results showed that the effect of the researched reactivators of the cholinesterase on the length of the hexobarbital sleep, microsomal mixed function monooxygenases and the quantity of the cytochrom P-450 and cytochrom b5, is a complex/multilevel process, and depends on the type and dose of the reactivator of the cholinesterase and the time of application. In general, the observed compounds showed tendency toward shortening of the hexobarbital sleep on the 2nd hour after their application. They do not change or lengthen it on the 24th hour, and they lengthen it after a 3-day treatment. The researched oximes – HS-6, HS-3 and HI-6, after application of various doses during later time intervals - 24 hours and mostly after the 3rd day of treatment, lead to suppression of the metabolism of some test-substrates of the cytochrome system, such as aniline. The conclusion is that most probably this is due to the effect of some of the metabolites of the above mentioned oximes, since it is well known also from the literature that the oximes are eliminated very quickly by the organism and this is done through metabolism.

SUMMARY

The reactivators of the ChE activity are almost always used with other drugs. Our previous research showed that toxogonine changes the liver drug metabolizing enzyme systems. We conducted investigations on Wister white male rats with b. weight 160-180g. (n=150) We made the analysis on the 2nd and 24th hour with HS-3, HS-6 and HI-6 with dosage 0,25 mmol/kg and 20 mg/kg b. weight i.m., as well as after three-day application of the same dosages.

Our results showed that the effect of the researched reactivators of the cholinesterase on the length of the hexobarbital sleep, microsomal mixed function monooxygenases and the quantity of the cytochrom P-450 and cytochrom b5, is a complex/multilevel process, and depends on the type and dose of the reactivator of the cholinesterase and the time of application. In general, the observed compounds showed tendency toward shortening of the

hexobarbital sleep on the 2nd hour after their application (HS-3 and HI-6). They do not change or lengthen it on the 24th hour, and they lengthen it after a 3-day treatment (HI-6). The researched oximes – HS-3 and HI-6, after application of various doses during later time intervals - 24 hours and mostly after the 3rd day of treatment (HI-6), lead to suppression of the metabolism of some test-substrates of the cytochrome system.

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KEY WORDES

H-oximes, HI-6, reactivators of ChE, liver, methabolizing enzime systems

FIGURES AND TABLES

Table 1. Effect of reactivators of ChE HS-3, HS-6, HI-6 on the hexobarbital sleeping time in male Wistar albino rats

	Sleeping time (min)		
	2 hours	**24 hours**	**72 hours**
Controls	24,00±6,70	20,00±4,90	
HS-6 (20 mg/kg)	25,20±8,00	24,20±5,21	
HS-6 (0,25 mmol/kg)	30,50±7,70	27,30±8,50	
Controls	26,50±3,70	22,00±0,63	
HS-3 (20 mg/kg)	18,30±1,33	23,00±1,79	
HS-3 (0,25 mmol/kg)	16,30±1,26*	25,60±1,69±	
Controls	23,80±1,32	23,00±0,41	17,70±2,00
HI-6 (20 mg/kg)	25,00±1,71	23,80±2,81	24,40±1,61*
HI-6 (0,25 mmol/kg)	18,00±1,30*	23,00±3,10	25,00±2,20*

* P<0,05

Table 2. The effect of 0,25 mM/kg b. weight HS-3, HS-6 and HI-6 on some rat liver microsomal parameters (2 hours after treatment)

	EMD nmol/mg.min	**AH nmol/mg.min**	**P450 nmol/mg**	**b5 nmol/mg**
Controls	2.24±0.11	0.235±0.015	0.44±0.05	0.21±0.05
HS-6	2.05±0.35	0.179±0.025	0.38±0.09	0.15±0.09
Controls	4.40±0.45	0.605±0.018	0.78±0.05	0.57±0.06
HS-3	3.93±0.35	0.723±0.019	0.66±0.08	0.38±0.06
Controls	2.45±0.19	0.149±0.01	0.43±0.01	0.17±0.02
HI-6	2.77±0.13	0.158±0.01	0.47±0.03	0.15±0.02

Table 3. The effect of 0,25 mM/kg b. weight HS-3 and HS-6 on some rat liver microsomal parameters (24 hours after treatment)

	EMD nmol/mg.min	**AH nmol/mg.min**	**P450 nmol/mg**	**b5 nmol/mg**
Controls	1.66±0.52	0.223±0.030	0.31±0.08	0.25±0.05
HS-6	1.90±0.31	0.230±0.032	0.38±0.09	0.33±0.06
Controls	3.96±0.41	0.545±0.024	0.84±0.07	0.42±0.03
HS-3	4.50±0.21	0.428±0.019*	0.99±0.05	0.52±0.01

Table 4. The effect of 0,25 mM/kg b. weight HS-3 and HI-6 on some rat liver microsomal parameters (3 days treatment)

	EMD nmol/mg.min	**AH nmol/mg.min**	**P450 nmol/mg**	**b5 nmol/mg**
Controls	4.97±0.66	0.508±0.009	0.54±0.04	0.30±0.03
HS-3	5.15±0.29	0.493±0.034	0.56±0.03	0.35±0.04
Controls	4.25±0.47	0.185±0.010	0.47±0.04	0.19±0.04
Hl-6	3.33±0.21	0.064±0.070*	0.43±0.03	0.26±0.02

* $P<0.05$